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APPENDIX

The mathematical treatment of Mechanism 1 given below is an extension of that given by Quast et al (1979) to include the fast relaxation, Step 2.

RATES

The fluorescence signal, F, at time t is proportional to the formation of $Y + C_1 + C_2$ where Y is equal to $R^*L + R^*L_2$. From Mechanism 1 the rates of change of the concentrations of these three species are given by the rate equations:

$$\begin{aligned} \dot{Y} &= k_2RL-(k_{-2}+k_3)R^*L + k_{-3}C_1 + k_{-5}C_2 - k_5R^*L_2 \\ \dot{C}_1 &= k_3R^*L-k_{-3}C_1 \\ \dot{C}_2 &= k_5R^*L_2-k_{-5}C_2 \end{aligned} \tag{A1}$$

where '•' indicates the derivative with respect to time. Assuming that the ligand binding steps are fast and can be considered as pre-equilibrium reactions to the isomerization steps and using the mass balance $R_o = R+RL+Y+C_1+C_2$ the rate equations can be rewritten, in matrix form as:

$$\begin{pmatrix} \dot{C}_1 \\ \dot{C}_2 \\ \dot{Y} \end{pmatrix} + A \begin{pmatrix} C_1 \\ C_2 \\ Y \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ \alpha R_o \end{pmatrix} \tag{A2}$$

where A is the reaction matrix

$$\begin{pmatrix} k_{-3} & 0 & -\gamma \\ 0 & k_{-5} & -\delta \\ \alpha-k_{-3} & \alpha-k_{-5} & \alpha+\beta+\delta+\gamma \end{pmatrix} \tag{A3}$$

and

$$\alpha = \frac{k_2 L}{K_1+L} \qquad \beta = \frac{k_{-2} K_4}{K_4+L}$$

$$\gamma = \frac{k_3 K_4}{K_4+L} \qquad \delta = \frac{k_5 L}{K_4+L} \tag{A4}$$

The calculation of the relaxation rates is simplified by the experimental fact that the three phases are kinetically well separated, i.e. $\lambda_F > \lambda_I > \lambda_S$. The characteristic polynomial ($|A-\lambda I| = 0$) for matrix A can be written in simple form as:

$$a_0 + a_1\lambda + a_2\lambda^2 + \lambda^3 = 0 \tag{A5}$$

where the coefficients, a_i, are rather complicated functions of the elements of the reaction matrix, A.

Assuming that the phases are all well separated the three relaxation rates may be approximated by (cf. Quast et al, 1979):

$$\lambda_F = a_2; \qquad \lambda_I = -\frac{a_1}{a_2}; \qquad \lambda_S = \frac{a_0}{a_1} \tag{A6}$$

These equations can be simplified by neglecting the terms which are relatively small in each equation and give the following equations:

(1) Fast Phase

$$\lambda_F \simeq \frac{k_2 L}{K_1+L} + \frac{k_{-2} K_4}{K_4+L} \tag{A7}$$

(2) Intermediate Phase

$$\lambda_I \simeq k_{-5} + \frac{k_5 \frac{L^2}{K_1K_2K_4}}{1 + \frac{L}{K_1K_2} + \frac{L^2}{K_1K_2K_4}} \qquad \text{(A8)}$$

(3) Slow Phase

$$\lambda_S \simeq k_{-3} + \frac{k_3 \frac{L}{K_1K_2}}{1 + \frac{L}{K_1K_2} + \frac{L^2}{K_1K_2K_4K_5}} \qquad \text{(A9)}$$

AMPLITUDES

Analysis of the amplitude data is simplified by the fact that the quantum yield change occuring in each isomerization is about equal. As described in the text the total amplitude is approximated by the relationship:

$$A_T = 2Q(\overline{C}_1 + \overline{C}_2) \qquad \text{(A10)}$$

the factor of 2 being introduced as a result of the fluorescence change occurcuring in the formation of R^*L.

The fluorescence signal at time t, F(t) is proportional to the concentration of $Y+C_1+C_2$:

$$F(t) = A_T - A_F \exp(-\lambda_F t) - A_I \exp(-\lambda_I t) - A_S \exp(-\lambda_S t) \qquad \text{(A11)}$$

The amplitude of each phase can be calculated using Equation (A1) from the boundary conditions:

$$F(0) = 0 = A_T - A_F - A_I - A_S$$

$$\dot{F}(0) = 0 = A_F\lambda_F + A_I\lambda_I + A_S\lambda_S \tag{A12}$$

$$\ddot{F}(0) = QR_o\alpha(\gamma+\delta) = A_1\lambda_F^2 + A_2\lambda_I^2 + A_3\lambda_S^2$$

This can be rewritten in matrix notation:

$$\begin{pmatrix} -1 & -1 & -1 \\ \lambda_F & \lambda_I & \lambda_S \\ \lambda_F^2 & \lambda_I^2 & \lambda_S^2 \end{pmatrix} \begin{pmatrix} A_F \\ A_I \\ A_S \end{pmatrix} = \begin{pmatrix} A_T \\ 0 \\ QR_o \end{pmatrix} \tag{A13}$$

Neglecting small terms of the order of λ_S^2 one obtains:

$$A_I = Q\frac{\lambda_S}{k_{-5}}\bar{C}_1 + Q\frac{\lambda_S}{k_{-3}}\bar{C}_2 \tag{A14}$$

for the intermediate amplitude and

$$A_S = Q[(1-\frac{\lambda_S}{k_{-5}})\bar{C}_1 + (1-\frac{\lambda_S}{k_{-3}})\bar{C}_2] \tag{A15}$$

for the slow amplitude. The two Equations (A14) and (A15) are identical to those derived by Quast et al (1979) which is not unexpected since for those calculations the fast phase (Step 2) can be considered to approximate a fast pre-equilibrium to the formation of C_1 and C_2. The fast phase amplitude can be obtained from the relationship:

$$A_F = A_T - A_I - A_S = Q[\bar{C}_1 + \bar{C}_2] \tag{A16}$$